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Looking for Inhibitors of RIO Kinases

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RIO kinases are atypical protein kinases involved in ribosome synthesis. The structure of RIO2 kinase was optimized. In particular, it was virtually titrated using a Poisson-Boltzmann model. An optimal protonation state was determined at pH 7 and pH 5. Screening of a ligand database against the target protein using a docking procedure was carried out. Small flexible deformations of a binding pocket were applied. Two possible inhibitors with the best scoring function are presented. The designed leading ligands and a number of their derivatives are being synthesized and will be studied experimentally.

1 Introduction

The RIO family (RIO1, RIO2, and RIO3) of atypical serine protein kinases is conserved among archaea and eukaryotes. At least two of them, RIO1 and RIO2 are present in these organisms[1,2]. Their involvement in ribosome synthesis, a process fundamental to cell growth and proliferation, makes them attractive targets for the development of inhibitors.

There are at least three subfamilies, RIO1, RIO2, and RIO3, and it was shown that the structural features of ATP binding pockets as well as the mode of substrate binding distinguish RIO1 and RIO2 kinases. Hence, given that there is only one copy of each RIO subfamily member per organism, this should allow to design inhibitors with high specificity, which can selectively target signalling/metabolic pathways RIO kinases are involved in.

2 Methods

Crystal structure of the RIO2 (1ZAO, PDB) kinase complexed with ATP, was used. All ligands (ATP, Mn and PO4 ions, and EDO (ethylen glikol) were removed. Missing fragments of loops residues (128-130 and 135-142), as well as missing side chains of the residues (26,41, 102,131, and 133) were modeled using MM and MD methods.

There are about 108 titratable sites in the protein (15 ASP, 27 GLU, 13 TYR, 8 IS, 21 LYS, 2 CYS, 20 ARG + termini). Protonation states of the residues were determined using a well established protocol which combines:

- the Poisson-Boltzmann model for a solute-solvent system with Monte Carlo calculations[3];
- the MEAD suite[4] is used to construct the electrostatic free energy matrices, describing interactions between protein's titratable residues in their charged states;

Titratable site	Occupancy	
	pH 7	pH 5
ASP 27	0.02	0.59
ASP 196	0.01	0.46
ASP 277	0.23	0.93
GLU 112	0.03	0.53
GLU 116	0.40	0.97
GLU 180	0.03	0.56
GLU 186	0.06	0.59
GLU 244	0.06	0.73
GLU 251	0.08	0.61

Table 1.

- the DOPS program[5] uses those electrostatic free-energy matrices to compute average protonation fractions at a given pH and to generate a predefined number of protein protonation patterns with the lowest energies as found by a Monte Carlo procedure.

Dielectric constants of the solvent and proteins were set to 80 and 4 respectively. Probabilities of protonation states were evaluated at ionic strengths corresponding to 150mM of monovalent salt, at pH values in the range between 5.0 and 8.0.

At pH 7, the protonation states are the regular ones, i.e., they correspond to the protonation states of free residues. However, the experimental condition of crystallization was an acidic one, below 5 pH. The way of protonation in such conditions changes significantly. In the former, the total charge of the protein is between plus 3-5, while in the latter, between 17-18. Significant changes of the protonation states were detected in nine residues (see Table 1) none of which, however, forms the ATP binding pocket.

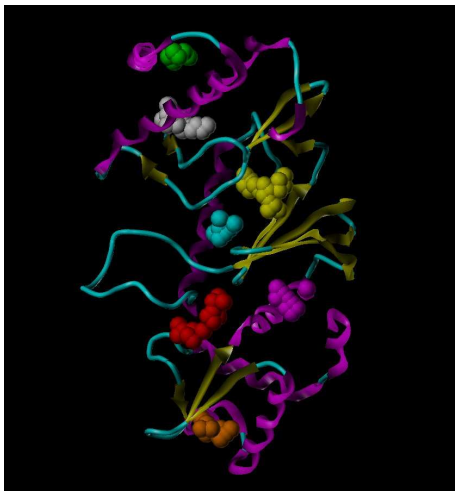
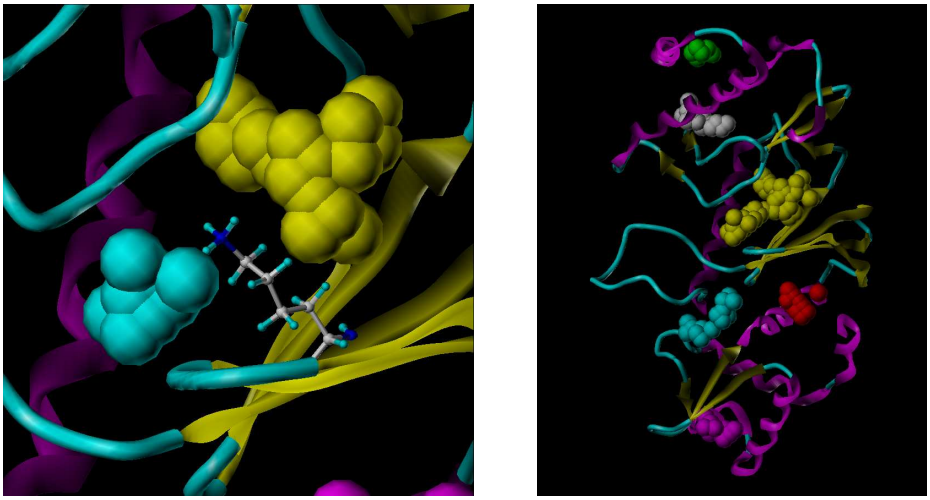


Figure 1. Location of 6 binding pockets (balls representation) of the RIO2 kinase.

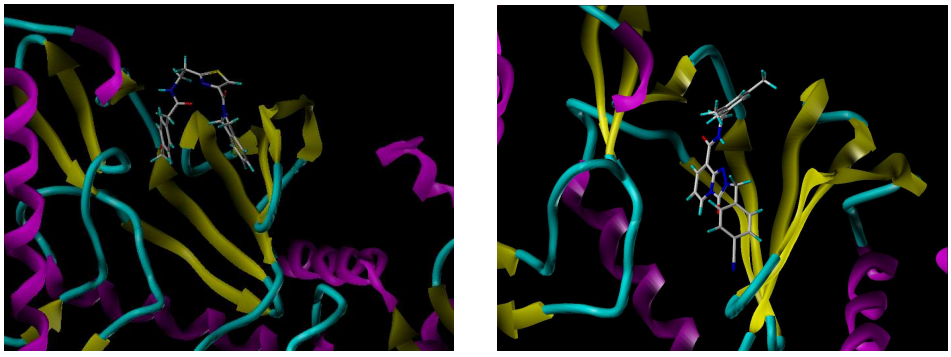


(a) (b)

Figure 2. LYS 120 side chain (a) protrudes into the binding site of the largest pocket of the RIO2 kinase (b), the yellow one.

3 Results

Possible pockets for the ligand binding are presented in Fig. 1. The ATP binding site is splitted by LYS 120 into two pockets (Fig. 2(a). These are yellow and cyan space-domains in the represetnation of the overlapping balls. Changes of the orientation of the LYS side chain results in formation of a much larger binding pocket shown in Fig. 2(b), the yellow one.



(a) (b)

Figure 3. Ligands with best score function for the RIO2 kinase.

Screening of ligands from LQ silver (`www.leadquest.com`) for the binding (Sybyl 7.3 Tripos Inc.) to the largest pocket were carried out. For comparison the non-modified two-region pocket was also scanned.

Ligand with the best score function for the non-modified position of the side chain of LYS 120 is shown in Fig. 3(a), and the best score ligand for the larger binding site is presented in Fig. 3(b).

4 Conclusions

- Novel potential inhibitors of the RIO2 kinase were designed.
- Optimization of the protonation states of ionizable side chains is of importance for the reliable modelling of RIO kinases and for the design of their inhibitors.
- Flexibility of the active site significantly changes its binding properties.

Acknowledgments

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